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Rainfall does not impair particulate matter accumulation on peri-urban field crops, but improves photosynthetic activity at UV exposure

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ABSTRACT

Anthropogenic aerosol pollution has intensified in (semi)-urban environments. Plant surfaces can accumulate a part of these aerosol particles in the terrestrial ecosystem. The deposition of aerosols on plant surfaces is a continuous process and is expected to be especially high in peri-urban agricultural environments. However, the deposited aerosols can be washed off by rain, or re-suspended by wind/rainfall, and rainfall is projected to increase due to climate change in some regions. In this study, we investigated how climate change, i.e. rainfall, affects the particles' deposition on field crops and how UV radiation can influence crop-particulate pollution interaction with respect to the photosynthetic, and antioxidant activity and their effects on plant health of the economically important cereal crop barley grown at field conditions near a peri-urban environment. Firstly, we quantified leaf surface particle accumulation and their elemental ionic concentration in ambient and rain-exclusion environments. Secondly, we observed the photosynthetic and antioxidant activity responses under UV and rain exclusion environments. Barley, having hydrophobic (water repelling) and narrow leaves, accumulated 0.5–1.0 µg (Na^+, K^+,Ca^{2+}, Cl^-) per square centimeter leaf area, whereas other measured ions (Mg^{2+}, $SO_4{}^{2-}$, NO^{3-}, $PO_4{}^{3-}$) were less than 0.3 µg cm^{-2}, and rainfall itself added a part of ions besides particles wash-off. Thus, about 19 rain events (> 5 mm d^{-1} precipitation, 5 times) did not affect long-term ionic mass accumulation on barley leaves. Aerosol accumulation at no rainfall and UV-exclusion reduced plant growth (i.e. plant height) by 20%. The results presented here suggest that leaf surface microstructures, area and foliage topography can affect aerosol accumulation irrespective of rainfall due to continuous deposition of aerosols. Rainfall can induce positive interactions between aerosol particles and crops at field condition, whereas aerosol accumulation together with UV exclusion during a rain-free season can affect their interaction negatively and impair plant growth.

1. Introduction

Leaf surfaces are the major receptor of aerosol deposition and accumulation of particulate aerosol (Neinhuis and Barthlott, 1998; Freer-Smith et al., 2005; Saebo et al., 2012; Burkhardt and Pariyar, 2014; Hofman et al., 2014). Since crops are grown on nearly 18 million km^2 (~12% of the cultivable land surface) (Leff et al., 2004), they can accommodate large numbers of particles from natural aerosols, air pollution, and agricultural sprays. The diameters of atmospheric aerosols are between a few nanometers and tens of micrometers, and many aerosols are hygroscopic, attract atmospheric humidity (Burkhardt et al., 2018) and act as cloud condensation nuclei in the atmosphere (Andreae and Rosenfeld, 2008; Burkhardt and Pariyar, 2016). Trees, compared to crop plants, are more efficient at capturing particles from the atmosphere by dry deposition (Gallagher et al., 1997; Mcdonald et al., 2007) and are considered as efficient pollutant filters in outdoor environments (Rai, 2016). In contrast, potted plants are considered as biofilters in indoor environment (Irga et al., 2018). Moreover, most of the anthropogenic particles originating from urban or peri-urban areas may be deposited on crop plants grown at the periphery, as dry as well as wet deposition (Poschl, 2005). However, particle deposition may be influenced by weather factors (rainfall, wind, humidity), and plant-species, shape and (leaf) surfaces (Przybysz et al., 2014; Chen et al., 2017; Hofman et al., 2017; Pariyar et al., 2017; Xu et al., 2017; Kardel

Abbreviations: AL, actinic light illumination; Chl *a*, *b*, chlorophyll *a*, *b*; DM, dry mass (g); ETR, electron transport rate (µmol m^{-2} s^{-1}); FM, fresh mass (g); Fv/Fm, maximum photochemical efficiency; NPQ, non-photochemical quenching; OD, outdoor (natural environment); PAR, photoactive radiation (µmol m^{-2} s^{-1}); PM, particulate matter; ROS, reactive oxygen species; RS, rain exclusion shelter

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et al., 2018; Liu et al., 2018).

Fine particles (particle diameter < 2.5 μm) are mostly hygroscopic (Pariyar et al., 2013), can create microscopic leaf wetness or form water films on plant surfaces (Burkhardt and Hunsche, 2013; Burkhardt et al., 2018). The microscopic leaf wetness can form continuous thin layers on hydrophobic leaf surfaces and may enable a strong potential influence on the foliar exchange of ions. It may also enhance the dissolution, the emission, and the reaction of specific atmospheric trace gases e.g., ammonia, SO_2, or ozone, leading to a direct plant/atmosphere interaction (Burkhardt and Hunsche, 2013). Thus, hygroscopic particles of the particulate pollution is a very important fraction in respect to the ecophysiological point of view, which may alter leaf photosynthetic efficiency in the changing environment. Crop adaptation to changing environmental factors involves both short-term physiological responses and long-term physiological, biochemical and structural modifications. These changes help plants minimize stress and maximize use of internal and external resources (Rai, 2016). Environmental changes due to atmospheric particulate matter (PM) pollution in (semi)-urban areas have exerted a profound influence on the morphological, biochemical, physiological and genetic status of plants (Rai, 2016).

The impact of PM on forest trees in terms of both physiology and biochemistry has been under research for some decades (Fluckiger et al., 1977; Burkhardt et al., 1995; Burkhardt and Pariyar, 2014; Hofman et al., 2014). Some of these studies have already demonstrated that leaf surface particles might have an effect on plant water relation and reduce drought tolerance (Fluckiger et al., 1977; Takamatsu et al., 2001; Burkhardt and Pariyar, 2014). There are only few studies, which have been undertaken on field crops. However, these studies were confined to mostly controlled conditions (Pariyar et al., 2013; Grantz et al., 2018), where integrated effects of UV and rainfall are lacking. Furthermore, rainfall effect on PM retention on plant surface is still a matter of debate and UV radiation has been considered as an abiotic stress factor. On the other hand, some studies reported that low dose of UV_B has positive effects on plants by increasing their antioxidant activity, thereby increasing their abiotic stress tolerance (Csepregi et al., 2017; Thomas and Puthur, 2017). Hence, more detailed investigations are necessary to characterize and quantify PM effects on field crops grown near polluted areas (urban or urban background) under a changing climate (precipitation and UV radiation). Therefore, we hypothesized that

i) the occurrence of a few to less frequent rain events may not alter aerosol particles accumulation on field crops,
ii) rainfall may improve the integrated effects of UV-radiation and PM accumulation on barley crops.

In this study, we show how climate, i.e. rainfall, affects the particles' deposition on a field crop and how rainfall and UV radiation can influence crop-particulate pollution interactions such as photosynthetic, and antioxidant activity and their effects on plant growth of the economically important cereal crop barley grown at field condition near a peri-urban environment. Firstly, we quantified the leaf surface particle accumulation during summer months and their elemental ionic concentration at ambient and rain-exclusion environments. Secondly, we observed the photosynthetic and antioxidant activity responses under UV and rain exclusion environments to compare how barley plant growth can be affected by PM, UV and rain.

2. Material and methods

2.1. Study site and crop growth condition

The experiment was carried out in 2017 at peri-urban field conditions at the research station of Campus Endenich, University of Bonn (50° 73.09' north latitude, 7° 7.34' east longitude), north-west of the city Bonn. Bonn is located at the river Rhein valley, where many motorways and transport ships cross by. It is considered as a moderately polluted city and trans-regional air pollution or even see salt might expected to reach to Bonn through the Rhein valley. The research field site is located closely to the motor highway A565, where around 55,000 vehicles per day passed by in 2015 (Federal Highway Research Institute, Germany). Barley (*Hordeum vulgare* cultivar 'Sissy') was grown in well-watered (water holding capacity above 50%) and nourished soil media (pot with basal-diameter 11 cm, soil mixture: TKS [Brill Typ 5 + sand + perlite, mixing ratio 1:1.2:0.3], water holding capacity: 1.29 kg water kg^{-1} DM). Barley seeds were sown directly into the soil pots (15 pots in each growth environment, pot-to-pot distance 10 cm) at field conditions. Only one plant per pot was allowed to grow after germination to avoid nutrition, soil moisture and light competition. Additionally, soil moisture content was recorded by SM150 moisture sensor (Delta-T-Devices, Cambridge, England) after a rain event to evaluate the soil moisture status after rainfall for the similar soil substrate and growth conditions in 05/07/2018. Each plant developed up to 4 tillers at the later stage of the vegetative growth. Potted plants were grown in two growth conditions; i) outdoors under ambient field conditions (OD; aerosol + rain + UV_{AB}), and ii) under rainfall exclusion, i.e. semi-transparent polythene shelter (RS; aerosol – rain – UV_B). The distance between the growth conditions (OD, RS) was less than 3 m at the same location (Supporting information 5). The rain shelter allowed free airflow from the two open longitudinal sides (east and west) at the bottom and ventilation (window) at the top vertical sides (south) (Supporting information 5). The open sides at the bottom and ventilations at the top of the shelters expected to be allowed equal particle deposition as ambient, and regulated temperature and relative humidity but excluded rainfall. Climate data such as global radiation, UV_A, rainfall and photoactive radiation (PAR) in ambient OD condition was continuously recorded (every minute) at the meteorological station (MWS 9-5 Microprocessor Weather Station, Rheinhardt System- und Messelectronic GmbH, Diessen-Oebermuelhausen, Germany) in the field, whereas plant canopy level temperature (T) plus relative humidity (RH) was continuously recorded (every 10 min) by Tinytag data loggers (Tinytag, Gemini Data Loggers, Chichester, UK); atmospheric air vapour pressure deficit at plant canopy (VPD, kPa) was calculated based on measured T and RH. Plant canopy level aerosol particles (counts, density) were discontinuously recorded by low-cost (not EU or EPA certified) air quality mobile sensors (LaserEgg, Origins Technology Ltd, Beijing, China), which use light-scattering (laser diffraction) principles to measure particles between 0.3 and 10 μm in an aerodynamic diameter. It converts the signal to a particle mass concentration and quantifies the resulting $PM_{2.5}$ concentration (μg m^{-3}) in 100 cm^3 air. The particle counts and density are recorded at 30 s time intervals. Additionally, the mass accumulation of particles in both OD and RS conditions were quantified during the rain free period (24/07–07/08/2018) to evaluate whether the PM deposition was similar to both growth conditions (i.e. OD and RS). Briefly, the clean and dry glass petridishes (area = 5.10 cm^2, n = 8) were weighed with a digital balance (1 μg sensitivity) and placed in the OD and RS conditions. After 15 days, the weight of the petridishes was taken to calculate the total mass depositions of particles. The calculated mass was then converted to mass per area (μg cm^2). PAR or UV_{AB} were discontinuously recorded by an Optometer (Gigahertz-Optik GmbH, Tuerkenfeld, Germany). The optometer measures photoactive radiation (PAR) (400–700 nm), UV_A (320–400 nm) and UV_B (290–320 nm) with calibrated PAR and UV sensors.

2.2. Microscopic observation and epicuticular wax quantification

Leaf surface micrographs of barley adaxial leaf sides (4th leaves from base, 21 days old, sampling on 21/06/2017) were recorded without conductive coating by a scanning electron microscope (SEM) (LEO 1530, Carl Zeiss AG, Oberkochen, Germany) on dry samples. SEM pictures were recorded at 1 kV. Since, SEM images recorded on dry

samples did not show clear leaf surface morphology due to shrinkage and desiccation of leaf tissues, SEM micrographs were also recorded on fresh leaves. In order to show clear leaf surface morphology, fresh leaves (16 days old) grown under growth chamber was used. The pictures were recorded by a scanning electron microscope with environmental control (XL 30 FEI-Phillips, Eindhoven, Netherlands), according to the method described earlier (Burkhardt and Pariyar, 2014). The pictures were recorded at 0.6 Torr.

Epicuticular waxes were extracted from adaxial sides (4th leaves from base, 26 days old, sampling on 26/06/2017) from each plant. Two ml of chloroform was filled in wax extraction glass containers and each selected area of the leaf (adaxial side) was brought in contact with chloroform for 10 s. The chloroform extract was further analysed for wax quantification. Epicuticular waxes were quantified by using gas chromatography and mass spectrometry analysis of all chloroform extracts based on the method previously reported (Zeisler and Schreiber, 2016).

2.3. Rain water and leaf surface ion analysis

The rainwater samples were taken on 6th June and 10th July 2017. A clean rain gauge was placed at the canopy level near the outdoor plants. After taking the rainfall reading on these two sampling days, the collected rainwater was used for ion analysis. The leaf washing ion samples were taken on 11th July for barley (30 days old, 6th position leaf from base, a topmost leaf per sample, 4–5 biological replications). The leaves were harvested, placed in clean and dry centrifuge tubes (15 ml volume, one leaf sample per tube) and brought to the lab. Since ion accumulation also depends on the leaf age and the main tiller of the barley plants had just 6–7 leaves in total from the base to the inflorescence (flower), one same age leaf sample per plant was assumed as representative for the study. The sample leaf was first thoroughly rinsed by 7 ml of deionised water in the tube. Then the leaves were dipped into it and shaken for 25 s in the rinsed water. The leaves were then taken out; photos were taken with mm-paper to calculate leaf area with the image processing software, ImageJ. The amount of water (7 ml) was equivalent to 1.5 mm rain amount collected in the rain gauge on volume basis, which was half of the mean precipitation (3 mm) amount during the experimental period. By rinsing and shaking, all the particles and ions were expected to be washed off or dissolved. For all ion analyses, the wash solution or rainwater was stored in a freezer (−20 °C) until the analysis was done. Before analysis, the solution was centrifuged (10,000 rpm, 5 °C, 5 min) to separate the solid fraction in the solution and then dissolved ions were analysed. The ion concentrations (μg L^{-1}) were quantified by ion chromatography (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) and atomic absorption spectrometry (Mg^{2+}, Na^+, K^+, Ca^{2+}). Ion concentrations (μeq L^{-1}) and depositions per leaf area (μg cm^{-2}) were calculated. Micro equivalent (μeq) was calculated as (μeq L^{-1} = equivalent molecular weight (mol mg^{-1}) * measured concentration (mg L^{-1}) * 1,000,000).

2.4. Measurement of photosynthetic activity

To evaluate whether different environmental factors (aerosol, UV, rainfall) influence the photosynthetic activity of barley leaves, the chlorophyll fluorescence measurement of barley leaves was done by pulse-amplitude modulated (PAM) chlorophyll fluorometry combined with saturating pulse analysis of fluorescence quenching. This measurement provides quantitative information on the quantum yield of photosynthetic energy conversion (Schreiber and Klughammer, 2008). The induction curves were recorded on 30th June for barley (4th position leaves from base, 30 days old) at laboratory conditions (ambient CO_2 concentration) using an imaging PAM fluorometer (ImagingPAM, Heinz-Walz GmbH, Effeltrich, Germany), equipped with 96 blue light-emitting diodes (peak at 470 nm) used for fluorescence excitation, actinic illumination and saturation pulses. Fluorescence images (640 × 400 pixel) were taken on the fully expanded leaves with one measurement per plant. Plants were dark-adapted (30 min) prior to the measurements. For each measurement, one representative (rectangular) area of interest (AOI) was selected. The recorded images were analysed by ImagingWinv2.46i (Heinz-Walz GmbH, Effeltrich, Germany). Ground fluorescence (Fo) was recorded after leaf illumination by the blue light-emitting diodes (0.5 μmol m^{-2} s^{-1}); the maximum fluorescence (Fm) was determined after a blue light saturation pulse of 1000 μmol m^{-2} s^{-1}. The yield of variable chlorophyll fluorescence (Fv) was calculated as Fm-Fo, while the maximum photochemical efficiency of the photosystem II (PSII) was calculated as Fv/Fm. After the first saturation pulse, actinic light (40 μmol m^{-2} s^{-1}) was switched on and saturation pulses (1000 μmol m^{-2} s^{-1}) were applied at 20 s interval within a period of 315 s. The other photochemical stress response parameters such as, quantum yield of regulated energy dissipation in PSII, Y(NPQ), quantum yield of non-regulated energy dissipation in PSII, Y(NO) and, apparent rate of photosynthetic electron transport (ETR, μmol m^{-2} s^{-1}), were quantified.

2.5. Measurements of leaf chlorophyll content and antioxidant activity

Five leaf samples from different plants for chlorophyll content (chlorophyll *a*, *b*) and antioxidant activity (proline accumulation) were taken on 11th July for barley (6th position leaves from base, 30 days old (exposure).

Chlorophyll *a* and *b* were quantified photometrically according to methods described previously (Holden, 1976; Strobl and Turk, 1990; Hoffmann et al., 2015). First, freeze-dried leaf samples were grinded. For the extraction, the grinded barley leaf samples were weighed (30 mg) and put into a 2 ml Eppendorf cup (Eppendorf GmbH, Wesseling, Germany). One ml methanol was added to each sample. The samples were shaken and centrifuged (13,200 rpm, 10 min, 4 °C). The extraction was repeated for five times until the extracts were colourless. The supernatant solution from each extraction (5*1 ml) was transferred into a 25 ml glass flask, and the 25 ml volume was adjusted with methanol. Absorbance (A) of the samples was measured at the wavelengths 650 nm and 665 nm with a UV/VIS spectrophotometer (Lambda 35, Perkin-Elmer, USA) using dissolvent resistant polystyrene cuvettes. Chlorophyll contents (Chl_*a* and Chl_*b*) were calculated according to the equations below.

Chlorophyll *a* (μmol g^{-1} dry matter): $[(16.5 \times A_{665nm}) - (8.3 \times A_{650\,nm})] * V/[M(Chl_a) * (Wt(sample))]$ (1)

Chlorophyll *b* (μmol g^{-1} dry matter): $[(33.8 \times A_{650\,nm}) - (12.8 \times A_{665\,nm})] * V/[M(Chl_b) * (Wt(sample))]$ (2)

Where, A = Absorbance of solutions at subsequent wavelengths (650 nm and 665 nm), V = volume of solvent (25 ml), M (Chl_*a*/Chl_*b*) = molar mass of chlorophyll *a* (893.51 g mol^{-1}) or chlorophyll *b* (907.49 g mol^{-1}) Wt = weight of the sample used for analysis (mg), Specific absorption coefficients with methanol extraction for chlorophyll *a* = 16.5 and 8.3, and for chlorophyll *b* = 33.8 and 12.8 (Holden, 1976).

Proline accumulation as a measure of antioxidant activity was quantified photometrically according to methods described earlier (Bates et al., 1973; Dolatabadian et al., 2008). The freeze-dried leaf samples were grinded. For the extraction, the grinded leaf samples were weighed (50 mg per sample) and 3 ml of 3% sulfosalicylic acid was added to each sample. The samples were shaken for 20 s and centrifuged at room temperature (4200 rpm, 20 min). Then 1 ml of supernatant solution was mixed with 1 ml of sulfosalicyclic acid in a clean test tube. Two ml of glacial acetic acid (100%) and ninhydrin acid reagent (1.25 g ninhydrin mixed in 30 ml of acetic acid and 20 ml of 6 mole phosphoric acid) were added to it, closed and shaken. The solution remained in the water bath for 1 h at 100 °C. After cooling the solution in an ice-bath to room temperature, 4 ml toluene was added to

the solution, the test tube was closed with a rubber plug and shaken vigorously for 30 s. After leaving the solution for 15 min, two distinctly different phases developed. The red coloured organic phase (upper part) was carefully pipetted in a half micro-acryl cuvette and the absorbance of the solution was measured in the photometer at a wavelength of 520 nm. The final proline concentration (μeq g^{-1} dry matter) was calculated according to the equation below, where the constant factor (s) is based on a proline standard curve.

$$\text{Proline (μeq g}^{-1}\text{ dry matter)} = [(A_{520\,nm} - b)/a) * V * DF]/[M(\text{proline}) * Wt(\text{sample})] \quad (3)$$

Where, $A_{520\,nm}$ = Absorbance of solutions at wavelength (520 nm), *a,b* = coefficients of a slope-intercept form of a linear equation ($y = ax + b$) of the standard proline concentrations gradient curve. V = solvent volume (3 ml), DF = dilution factors = 2 (2 ml/supernatant), M (proline) = molar mass of proline (115.5 g mol^{-1}), Wt = weight of the sample used for analysis. μeq = micro equivalent (= equivalent molecular weight (mol mg^{-1}) * measured concentration (mg L^{-1}) * 1,000,000).

2.6. Plant growth and biomass

Plant height was recorded at 46 days after sowing and shoot fresh mass (FM) or shoot dry mass (DM) at harvest were quantified at 53 days after sowing. After measuring the FM, the samples were oven dried at 60 °C for 72 h and weighed again to measure DM.

2.7. Data analysis

Statistical analysis was performed using Sigma Plot (v. 13) and SPSS (v.25). For plant samples, data was checked for normality (Shapiro–Wilk test) and homogeneity of variance (Brown-Forsythe test). If both tests were passed, a two-tailed Student's *t*-test was performed. When normality failed, a two-sided Mann–Whitney-*U* test was used to evaluate between the two groups. In all statistical analyses, differences were considered significant if $p < 0.05$.

3. Results and discussion

3.1. Plant growth environment

The ambient air around the experimental location usually contained 10,000–20,000 particles cm^{-3} (particle size > 0.1 μm) (Burkhardt and Pariyar, 2016). The following air particles (mean ± SE) in the experimental site on the selected dates and times were measured: i) particles > $PM_{2.5}$ were about 0 to 3 ± 1 in both growth conditions (OD, RS), except it was higher for RS just once during measured dates (Supporting information 1). ii) fine particles ($PM_{2.5}$) were about minimum of 3 ± 1 μg m^{-3} after 6 mm rain on 7th July 2017 and maximum of about 52 ± 2 μg m^{-3} on a moderately polluted day on 6th July 2017. $PM_{2.5}$ did not differ on some days, was higher for OD between 8–31% in 2 measurement days and 20% higher for RS in one measurement day (Supporting information 1). iii) total particle numbers greater than 0.3 μm were about minimum of 721 ± 55 after 6 mm rain on 7th July 2017 and maximum of about 6965 ± 183 on a moderately polluted day on 6th July 2017. OD had about 9–13% higher total number of particles as compared to RS, except on one day (6th July 2017, Supporting information 1). However, the higher number of ultrafine particles might have had a negligible contribution on particle mass. Additionally, the total particle mass accumulations during 15 days at no rainfall in OD was 154 ± 11 μg cm^{-2} (mean ± SE) and in RS (196 ± 47) μg cm^{-2} (mean ± SE), and did not differ significantly ($P > 0.05$, n = 8) (Supporting information 2). However, higher (~20%) particle mass accumulation in RS during this period was due to course solid particles, possibly solid black carbon particles (Supporting information 2: picture) and may not contribute to the ionic mass accumulation. The reported number of particles on a moderately polluted day (6th July 2017) was lower than the earlier report. It should be noted that the measurement was done 1 m above ground, one day after 1 mm rain, and the counted particle size was > 300 nm. At the same day and time, PM_{10} (particulate matter with aerodynamic diameter < 10 um) concentration at Bonn–Auerberg (60 m above ground, 2 km away from the experimental site) was 17 μg m^{-3} (LANUV – NRW, Germany).

Table 1
Meteorological parameters recorded in the field experiment site, where barley (*Hordeum vulgare*) plants were grown either outdoors (OD) or in a rain exclusion shelter (RS) in the field. Data are mean ± SE, n = 54[a,b] (mean of each day is a replication during growth period 18.05–10.07.2017).

Climate and environmental parameters	Plant growth conditions	
	OD	RS
Global radiation (W m^{-2})[a]	328 ± 14	n.a.
PAR (μmol m^{-2} s^{-1})[a]	554 ± 20	n.a.
UV_A (W m^{-2})[a]	5.0 ± 0.2	n.a.
Rainfall (mm)[a]	3.5 ± 0.8 29 rain events (> 5 mm, 9 times)	0
T (°C)[b]	23.7 ± 0.5	23.4 ± 0.5
RH (%)[b]	61.4 ± 1.4	53.3 ± 1.3
VPD (kPa)[b]	1.2 ± 0.1	1.4 ± 0.1

n.a. = data not available.
[a] Weather station (1.5 m above ground).
[b] Crop canopy (1.0 m above ground).

The particle concentrations in air decreased after every rainfall and after a while increased again depending on the time of the day, wind and pollution events (Supporting information 1). During the growth period (18/05–10/07/2017), PM_{10} concentration at Bonn–Auerberg was 22 ± 0.4 (mean ± SE) μg m^{-3}, reaching a maximum of 48 μg m^{-3} (LANUV-NRW, 2018). The global radiation, photoactive radiation (PAR) and UV_A in the OD environment were 328 ± 14 W m^{-2} (mean ± SE), 554 ± 20 μmol m^{-2} s^{-1} and 5.0 ± 0.2 W m^{-2}, respectively (Table 1). The temperature and vapour pressure deficit were the same for both OD and RS; however, relative humidity in OD was about 13% higher than RS (Table 1). Canopy level PAR was almost same (less than 3% deviation) for both OD and RS at cloudy or low radiation condition, except at very sunny and clear middays (19% higher for OD) (Supporting information 3). Similarly, UV_{AB} in OD were minimum about 8 W m^{-2} (low radiation condition) and maximum about 48 W m^{-2} (very sunny midday), whereas in RS were min. about 3 W m^{-2} (low radiation condition) and max. about 8 W m^{-2} (very sunny midday), respectively (Supporting information 3). RS plants received about 38% and 80% lower UV_{AB} at low and high radiation conditions than OD plants, respectively. In addition, RS plants received 92–99% less UV_B as compared to OD plants (Supporting information 3). RS plants did not receive rainfall at all (Table 1).

3.2. Rainfall vs. leaf surface ion accumulation

The 30 days old barley leaves received 19 rain events (leaf initiation to measurement day, > 5 mm d^{-1} - 5 times) ranging from 0.11 to 15.3 mm d^{-1} with a total average precipitation of 3 ± 1 mm (mean ± SE) and total precipitation sum of 55 mm during their growth period (11th June–10th July). The barley leaves accumulated 0.7 μg cm^{-2} (Cl^-) and 1.0 μg cm^{-2} (Na^+, K^+) ions within a month at ambient natural field conditions, including rainfall (Fig. 1c). In addition, precipitation itself might contribute to atmospheric ions deposition during rain events as wet deposition (Fig. 1d); however, the ion concentration was quite low. The concentration of most measured ions did not differ between the two growth environments, OD and RS

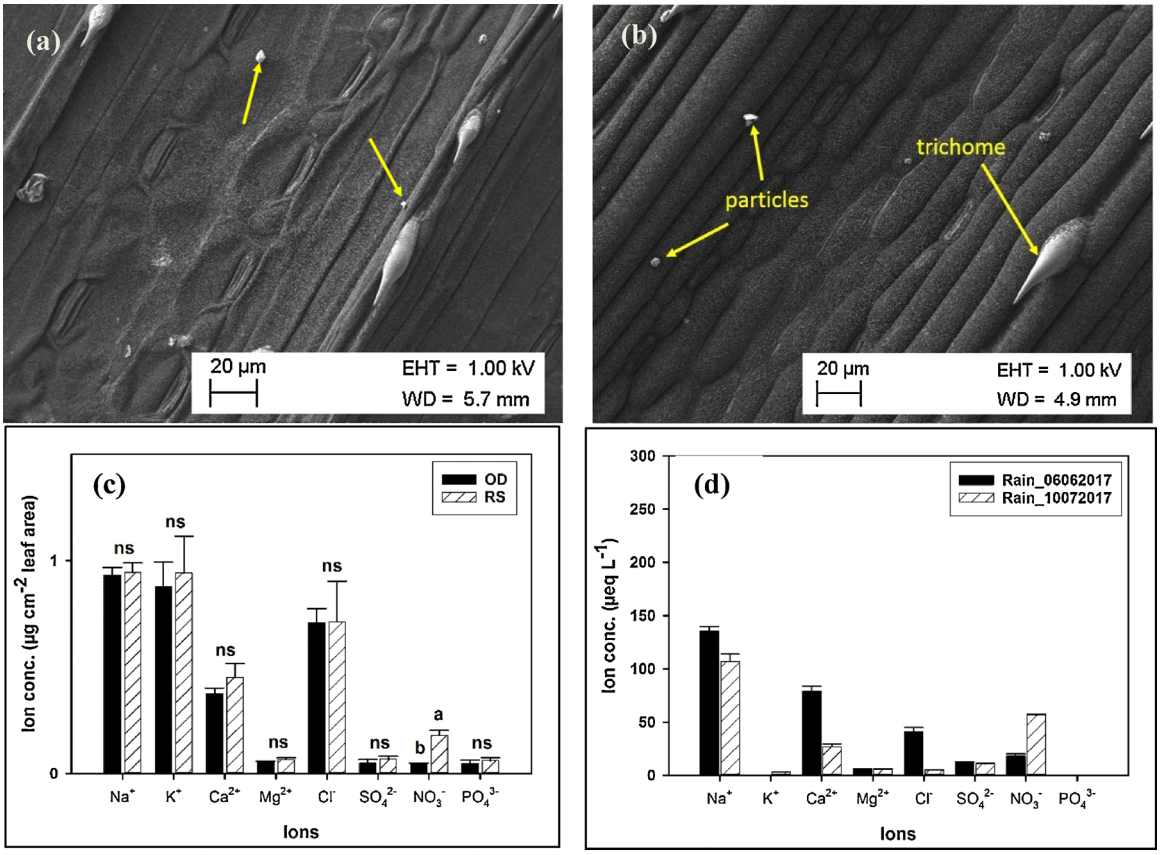

Fig. 1. Scanning electron micrographs showing the particles and trichomes (indicated by arrows) on barley adaxial leaves grown in ambient outdoor, OD, (a) or in rain exclusion shelter, RS, (b) conditns. (c) Leaf surface ion accumulation on 30 days old barley leaves (6th position from base, top-most leaves) grown in ambient field conditions, OD and RS. (d) Ion concentrations of rainwater collected at two different dates during experimental period. Bars: mean ± SE, n = 4–5 (biological replications). Significant differences are denoted by different letters (a, b) at $P < 0.05$. ns = non-significant.

(Fig. 1c). Some of the earlier reports suggest that simulated rain or water cleaning reduced particulate matter mass accumulation on shrub or tree leaf foliage by 20–45% compared to before rain or washing (Xu et al., 2017; Liu et al., 2018). However, in this study, natural rainfall did not influence ion accumulation at field conditions. This may be due to the continuous deposition at natural environments, even though rain-washing of ions occurred during precipitation for OD. In addition, some studies postulated that small or nano or magnetic dust particles are often not washed off significantly from leaves by rainfall (Freer-Smith et al., 2005; Hofman et al., 2014; Larue et al., 2014; Kardel et al., 2018). In addition, wet deposition of aerosols during precipitation can contribute to ion loading (Fig. 1d) besides wash-off of hygroscopic aerosols. In an OD environment, NO_3^- ion deposition was found to be significantly more reduced than in RS. NO_3- ion might have leached with precipitation in OD, while in the RS environment, it remained un-washed. Or, it may have been better absorbed into inner plant tissue by water films formed by rain in OD thus facilitating uptake of ions. The sum of the measured ions in OD was 3.4 µg cm^{-2} and in RS was 3.1 µg cm^{-2}, respectively. Barley leaves seemed to accumulate more ions than scots pine needles (2.1 µg cm^{-2}), but less than beech leaves (4.3 µg cm^{-2}), and Na^+ and Cl- were the dominating ions measured at a nearby (1 km apart) location (Burkhardt and Pariyar, 2016). However, particles accumulation on leaf surface depends on the atmospheric exposure, pollution level, leaf type, shape, size, topography, age and microstructures such as epicuticular waxes. While comparing between barley and scots pine, the leaf size and the microstructures might have influenced ions accumulation. Barley leaf has wavy convex cellular structures, very dense clustered wax plates, anchor shaped trichomes and, long, narrow and hydrophobic (water repelling) leaves. Besides surface morphology, the volatiles accumulation by plants on their surface might affect particle accumulation. Thus, it is difficult to conclude which species is more efficient at accumulating particles. Here, further studies should consider comparing species wise particle accumulation at the same environment.

3.3. Photosynthetic and antioxidant activity

The chlorophyll fluorescence measurement of barley leaves showed no differences in photosynthetic activity (ETR, electron transport rate, µmol $m^{-2} s^{-1}$) and regulated energy dissipation of PSII (NPQ, non-photochemical quenching) (Fig. 2a,b, Table 2). ETR indicates the apparent rate of photosynthesis. Although OD plants were exposed to higher UV radiation and PAR was almost similar to RS conditions (except a relatively higher PAR during sunny middays), there were no differences in ETR between OD and RS plants. We postulate here that the down regulation of electron transport at a higher PAR between two photosystem centres by higher UV might have influenced the ETR of OD plants. Natural UV radiation is considered a major environmental factor for UV inhibition on PSII (Pfundel, 2003). The energy regulation mechanism in the photosystems can be further elaborated by NPQ. On one hand, a high NPQ indicates that the photon flux density is excessive and on the other hand, it shows that the plant has retained the physiological mechanisms to protect itself by regulation, i.e. the dissipation of excessive excitation energy into harmless heat. Without such dissipation, there would be a formation of singlet oxygen and reactive radicals, which causes irreversible damage (leaf burn or necrosis). Since NPQ plays a major role in the protection of the photosynthetic apparatus against damage by excess light, which is closely associated to the energy redistribution in two photosystems and production of reactive oxygen species (ROS) (Hideg et al., 2008; Demmig-Adams and Adams,

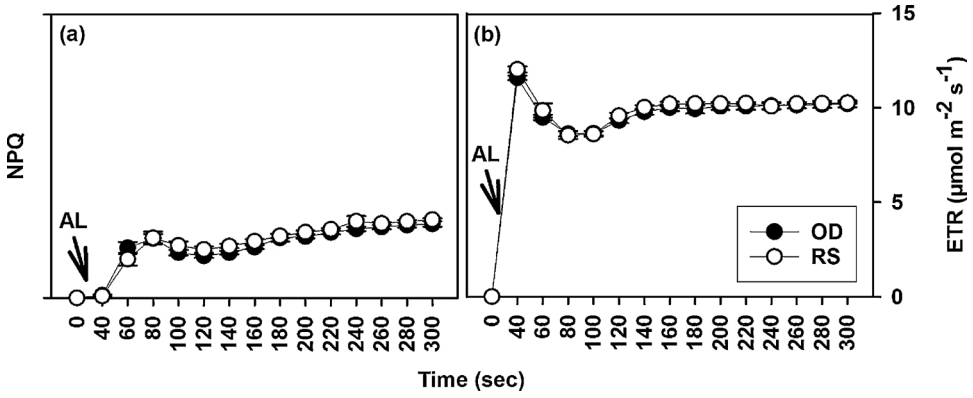

Fig. 2. Comparison of photosystem kinetics of barley (*Hordeum vulgare* cultivar Sissy) grown under ambient outdoor (OD) and rainshelter (RS) conditions. (a): non photochemical quenching (NPQ), which regulates the energy dissipation in PSII, (b): electron transport rate (ETR, µmol $m^{-2} s^{-1}$), which illustrates the photosynthetic activity in PSII. Fluorescence measurement was performed on the 4th position leaves, 30 days old and 30 days after exposure to OD or RS environments. AL = actinic light illumination. Values: mean ± SE (n = 7).

Table 2
Statistical comparison of photosystem reactions of barley dark-adapted leaves between ambient outdoor (OD) and rain shelter (RS) growth conditions. Each data point is the mean of seven biological replications either at dark or with saturated light pulse at constant actinic light (photoactive radiation: 40 µmol $m^{-2} s^{-1}$).

Parameters		Treatments	
		OD	RS
Maximum fluorescence yield at light saturation	Fm'	A	a
Photochemical efficiency of photosystem II	Fv/Fm	A	a
Coefficient of non-photochemical quenching	qN	A	a
	ETR	A	a
Quantum yield of regulated energy dissipation	Y(NPQ)	A	a
Quantum yield of non-regulated energy dissipation	Y(NO)	A	a
Absorptivity	Abs	A	b
Effective photosystem quantum yield	Y(PSII)	A	a
Sample size	n	15	15
Significance	Mann–Whitney *U* test ($P < 0.001$)		

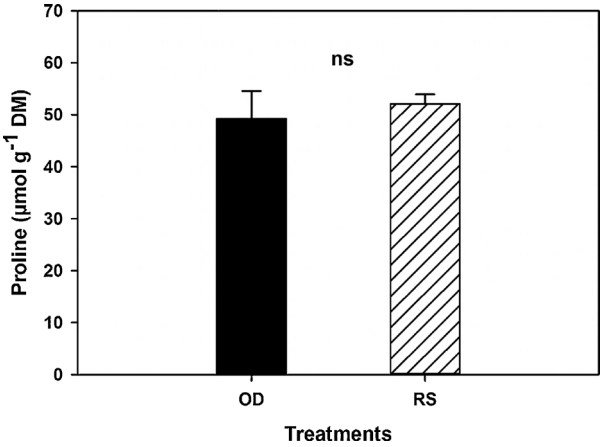

Fig. 3. Proline content of 30 day-old barley leaves (6th position from base) either grown in ambient outdoor (OD) or in rain shelter (RS) conditions. Bars: mean ± SE, n = 5. ns = not significant ($P > 0.05$, Tukey test).

2018; Foyer, 2018), OD plants might have increase antioxidant enzymes to combat oxidative damage against UV radiation. Earlier studies reported that the low level of UV_B exposure to induces accumulation of non-enzymatic antioxidants like proline, which enables plants to increase their stress tolerance (Csepregi et al., 2017; Thomas T.T and Puthur, 2017).

Proline is an amino acid that accumulates in plants in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals or pollutants (Ashraf and Foolad, 2007; Fallard et al., 2018), and plays a highly beneficial role in plants exposed to various stress conditions (Hayat et al., 2012). Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as an antioxidative defense molecule, a metal chelator and a signaling molecule (Hayat et al., 2012). In this study, proline accumulation is interpreted as a measure of antioxidant activity. The mean proline content was 54 ± 2 µmol g^{-1} DM and 52 ± 2 µmol g^{-1} DM for OD and RS barley plants, respectively. Proline accumulation did not differ between OD and RS (Fig. 3). This result could postulate that the conservation of proline may be related to the photosynthetic activity, where OD and RS plants have equal maximum photochemical efficiency (Fv/Fm) and ETR, and, a similar relationship was also reported earlier (Fallard et al., 2018). Proline concentrations of barley leaves are in the same range of barley genotypes at no-salt-stress conditions (Chen et al., 2007), but were quite high compared to other crops (apple, soybean) grown at the same condition (unpublished results). This result indicates that the barley crop (grass family) can retain stress tolerance mechanisms by increasing antioxidant activity better than some other crops like sweet potato (Yooyongwech et al., 2017).

3.4. Chlorophyll content and epiculticular wax

Leaf chlorophyll content differed significantly between OD and RS (Fig. 4a), which was also observed visually (Fig. 4b). The mean chlorophyll contents (mean ± SE, n = 5) were 8.9 ± 0.3 µmol g^{-1} DM for OD and 5.6 ± 0.7 µmol g^{-1} DM for RS, respectively. Total chlorophyll content (Chl *a*, *b*) was about 37% less for RS than OD. The decrease in chlorophyll content of RS plants could be due to the reduced UV and PAR exposure. RS plants received 38–83% lower UV and 0–19% PAR based on selected measurement dates and time.

Previous study reported that barley leaves are hydrophobic (water repellent), with high surface roughness, i.e. coverage of > 60% wax crystals, trichomes or both, very convex epidermal cells and many ridges (Wang et al., 2014). Our observation on leaf surface microstructure support this. The presence of anchor shaped trichomes, wavy (high and low) tubular cell structures, erect and dense wax crystals was observed on adaxial leaves (Fig. 4c, d). The wax microstructures were erect and composed of densely distributed clusters of wax plates (Fig. 4e, f) and were presence in almost all areas of the leaf surfaces, including trichomes. The wavy (high and low) tubular cell structures are similar to scots pine, whereas wax microstructure seemed to be different (barley: clustered plates, scots pine: tubular (Burkhardt and Pariyar, 2016; Burkhardt et al., 2018)). The presence of trichomes was observed in barley, but not in scots pine.

Amounts of epicuticular wax extracted from adaxial leaf surface were 21.1 ± 4.1 µg cm^{-2} and 12.7 ± 0.6 µg cm^{-2} leaf area (mean ± SE, n = 5) for OD and RS conditions, respectively. OD plants had around 42% more epicuticular waxes as compared to RS plants,

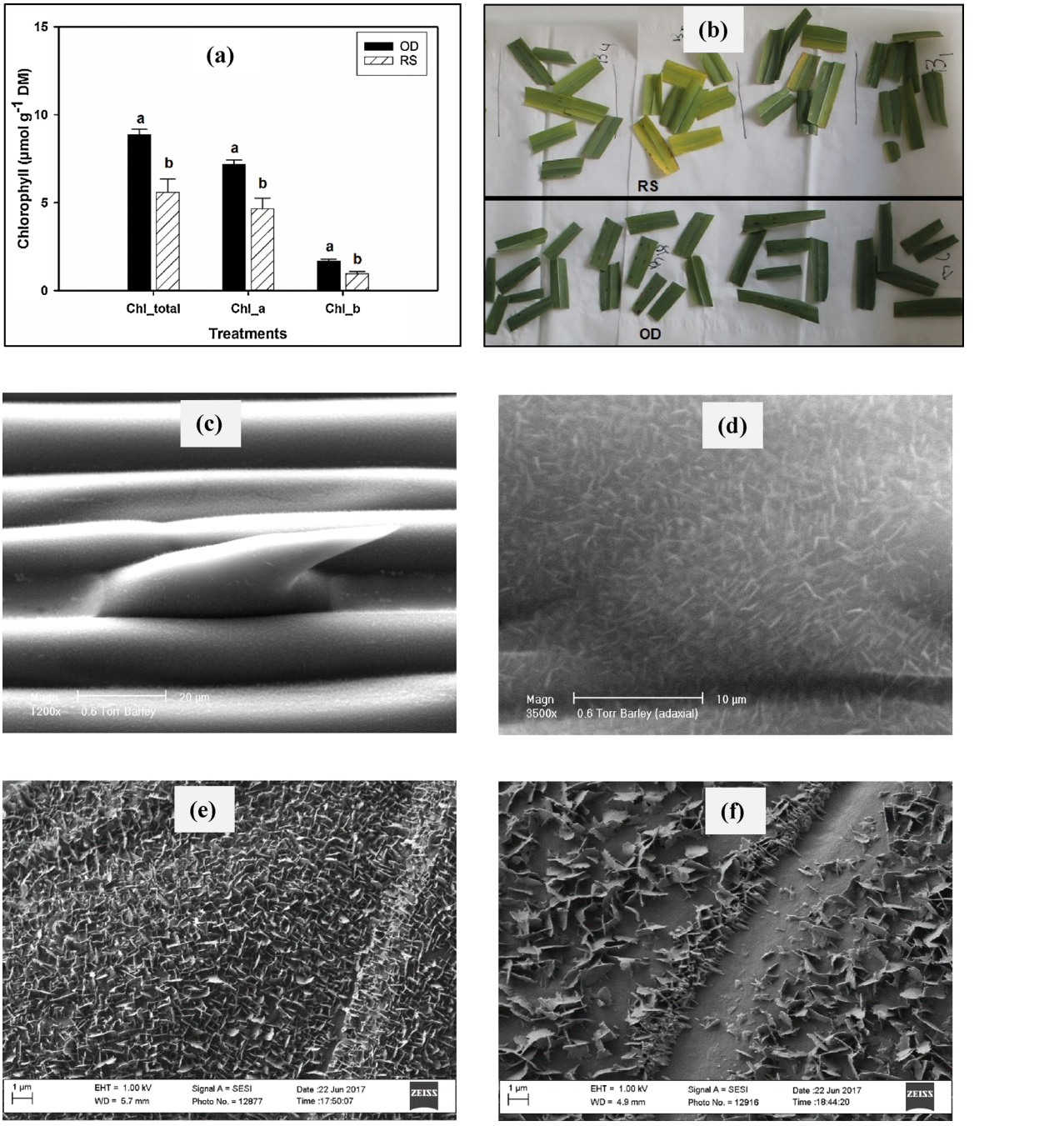

Fig. 4. (a) Chlorophyll content of 30 day-old barley leaves (6th position from base) either grown in ambient outdoor (OD) or in rain shelter (RS) conditions. Bars mean ± SE, n = 5. Significant differences are denoted by different letters (a, b) at $P < 0.05$. (b) Photo showing the visible differences in leaf chlorophyll between OD (lower part) and RS (upper part). Scanning electron micrographs: Barley leaf surface with a trichome (c) and wax microstructures (d) observed on fresh leaves (16 days old) grown in growth chamber, Wax microstructure and abundance on 10 days old barley leaf grown under OD condition (e) and under RS condition (f) observed in SEM.

which can also be observed in SEM micrographs (Fig. 4e, f). Although, previous studies demonstrated that the particulate pollution can have an impact on wax degradation on forest species (Huttunen et al., 1981; Burkhardt and Pariyar, 2014), the reduction in the amounts of epicuticular waxes of RS plants possibly occurred due to meteorological conditions (wind, rainfall, UV and/or global radiation). It is assumed that plants exposed to less stress have to build lower cuticular barriers in protecting plants from biotic and abiotic stresses as also mentioned in previous reports (Robinson and Osmond, 1994; Pollard et al., 2008; Fernandez et al., 2017).

3.5. Plant growth and biomass

Barley plant growth was different between OD and RS. OD plants were taller with less lateral leaves, whereas RS plants had more lateral leaves and were shorter. Thus, plant height significantly differed (Fig. 5a), which was also observed visually (Fig. 5b). Although OD plants had relatively higher FM and DM (10% and 13%, respectively), they did not differ significantly (Fig. 5c, d). The better plant growth in OD conditions possibly occurred due to i) the frequent cleaning of course particles by rainfall, which enabled light incidence on leaf chloroplasts and had no effect on chlorophyll pigments, as the

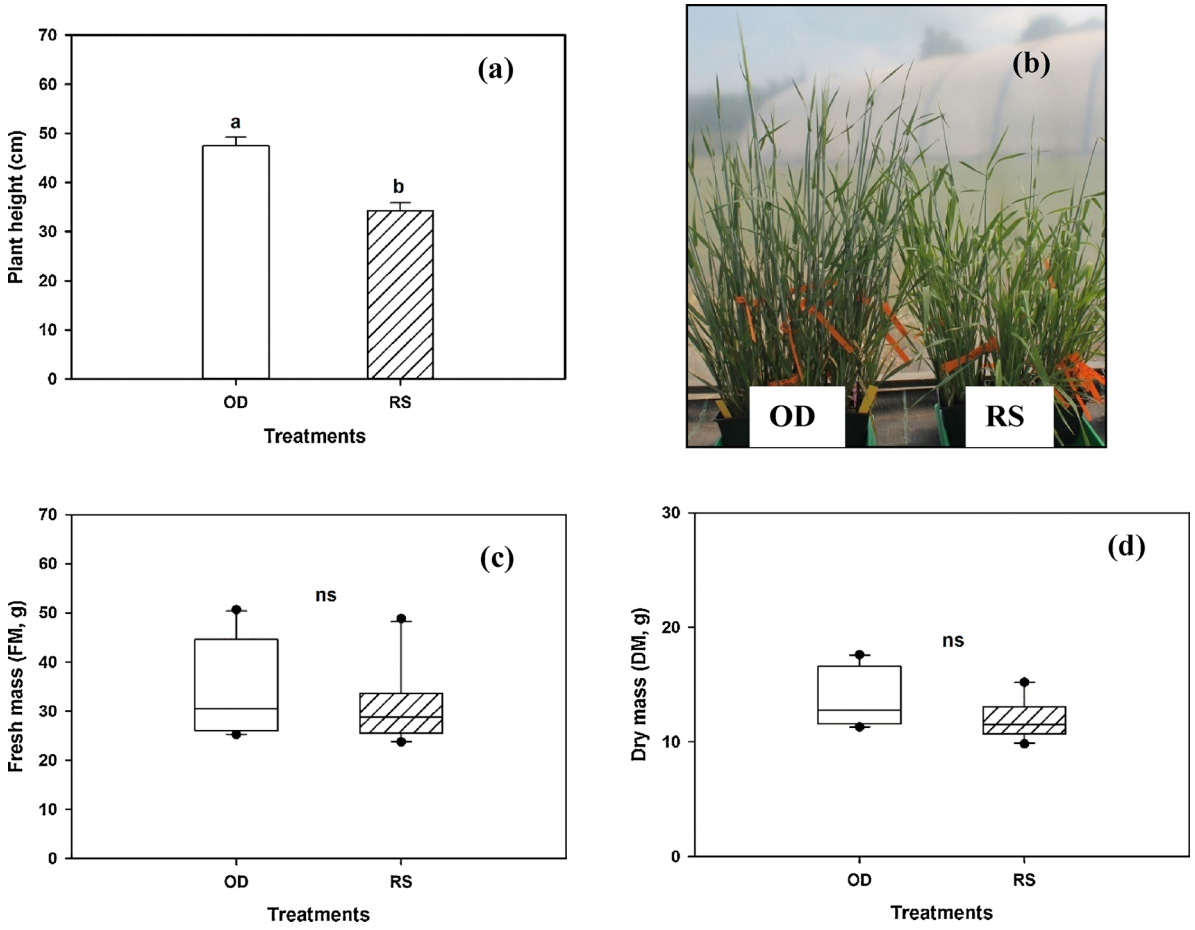

Fig. 5. (a) Plant height (cm) measured 46 days after sowing (DAS), Bars: mean ± SE, n = 55–60, P < 0.001. (b) Picture showing the barley (*Hordeum vulgare* cultivar 'Sissy') plants grown in ambient outdoor natural conditions (OD) and rain shelter (RS) at 35 DAS in respective growth conditions. (c) Fresh shoot biomass and (d) shoot dry mass were measured on 53 DAS. Box plot: median ± quartile (upper 25%, lower 75%), n = 10. ns = not significant (P > 0.05).

reduction in chlorophyll concentration is correlated to plant growth (Marinari et al., 2007; Rai, 2016). The presence of visibly smaller black particles in OD as compared to RS particles collectors (Supporting information 2, picture) may be an additional evidence. ii) nutrient ions (especially NO_3^-) may have been better absorbed into inner plant tissue by water films formed by rain in OD thus facilitating uptake of NO_3^- ions and better growth. iii) OD plants received rainfall during their growth, which could have partially contributed to the increase in solute concentrations via rainwater (Fig. 1d) or via seepage or infiltration from top layers and increased nutrients availability to roots due to soil becoming wet throughout the pot. One day after 1 mm rainfall, in addition to normal watering, the recorded soil moisture content near the root zone was not different between OD and RS environments (OD: 64 ± 2%, RS: 66 ± 2%, n = 11, P > 0.05) (Supporting information 4), while soil moisture was observed to be equally distributed. iv) PAR could have also partially contributed to the plant height, as OD plants received relatively higher PAR during high radiation periods of the day, even though it did not deviate most of the selected measurement days between OD and RS environments. v) presence of UV (especially UV_B at natural condition), even though plant responses to UV_B highly varied (Biever and Gardner, 2016). UV radiation may induce antioxidant (organic molecules) accumulation in plants; thereby they may enhance photo-protective functions and stress tolerance (Ashraf and Foolad, 2007; Hernández et al., 2009; Agati et al., 2013; Guidi et al., 2016). This can be proven by proline accumulation in leaves (for both OD and RS conditions), which correlates to the biomass as reported in previous study (Ueda et al., 2008).

4. Conclusion

This study suggests that barley, a field crop grown near a semi-urban environment, can accumulate substantial amounts of particulate pollution ions. These ions may be hygroscopic and may originate from coarse, fine and ultrafine particles. At outdoor field conditions, the deposited aerosols may not be washed off (or only partially washed off) from the hydrophobic surfaces of barley leaves by natural rainfall. In addition, particles (ions) deposition may occur continuously. Since barley has hydrophobic leaves with high surface roughness, the contact area or retention of rainwater droplets on barley leaf surfaces during precipitation might be reduced. This may affect ions dissolution, and rain wash-off might be less efficient on hydrophobic leaves. Thus, ion accumulation remains unchanged and rainfall does not impair particulate ion accumulation on barley grown near (semi)-urban environments. Similar tendencies of accumulation could be expected for other solid or heavy metal particles on barley leaf. In addition, we speculate that the dry and wet deposition on leaves may vary on particles accumulation and their localization on trichomes, ridges, waxes, veins, and cell walls. However, partitioning of hygroscopic and solids or heavy metals particles and their deposition patterns within the leaves/plant surfaces have to be further investigated. Furthermore, rainfall and UV exposed plants in an OD environment maintained photosynthetic and antioxidant activity similar to RS condition. The higher chlorophyll content for photo-energy conversion, possibly higher solute concentration due to rain and higher epicuticular wax as a protecting mechanism against environmental stress might have contributed to the better growth for OD plants. Thus, natural rainfall in an OD or ambient

environment might reduce the potential effects of UV and other environmental factors. Consequently, the aerosol pollution and UV radiation in a rain-free season (similar to RS environment) may affect barley crop growth negatively. However, the particulate matter accumulation and associated physiological effects may vary among field crops, thus further studies should consider evaluation of the specific genotypic responses to particulate pollution – climate interaction.

Author contributions

SP carried out the experiments, data collection and analysis. SP wrote the first draft. GN provided critical review and edited the manuscript. SP and GN reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2018.09.019.

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